



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 35/74	A1	(11) International Publication Number: WO 98/43655 (43) International Publication Date: 8 October 1998 (08.10.98)
(21) International Application Number: PCT/CA98/00297 (22) International Filing Date: 2 April 1998 (02.04.98) (30) Priority Data: 60/040,908 2 April 1997 (02.04.97) US (71) Applicant: BIONICHE INC. [CA/CA]; 383 Sovereign Road, London, Ontario N6M 1A3 (CA). (72) Inventors: ALKEMADE, Stanley, J.; 12801 Medway Road, R.R. #1, Arva, Ontario N0M 1C0 (CA). HILDEBRAND, Catherine, E.; 111 Garfield Avenue, London, Ontario N6C 2B7 (CA). PHILLIPS, Nigel, C.; 101 Seignior Avenue, Pointe-Claire, Quebec H9R 1J6 (CA). ROGAN, Dragan, R.; 138 Saddy Avenue, London, Ontario N5V 4N1 (CA). (74) Agent: CAMPBELL, Hugh, D.; Finlayson & Singlehurst, 70 Gloucester Street, Ottawa, Ontario K2P 0A2 (CA).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>With amended claims and statement.</i>
(54) Title: USE OF BACTERIAL CELL WALL EXTRACTS FOR TREATING TOPICAL DISORDERS AND WOUNDS (57) Abstract <p>A method for treating a topical disorder or a wound in an animal by administering a sufficient amount of a bacterial cell wall extract to an animal suffering from a topical disorder or a wound to reduce or eliminate the topical disorder or symptoms thereof, or to promote or accelerate wound healing. Preferably the bacterial cell wall extract is a mycobacterial cell wall extract prepared from a mycobacterium such as <i>Mycobacterium phlei</i>.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

5

1

1 0 USE OF BACTERIAL CELL WALL EXTRACTS FOR TREATING TOPICAL DISORDERS AND WOUNDS

CROSS REFERENCE TO RELATED APPLICATIONS

1 5 This application claims priority to U.S. Provisional Application No. 60/040,908 filed April 2, 1997.

FIELD OF THE INVENTION

2 0 The present invention relates to the fields of dermatology and immunology and more particularly relates to the therapeutic use of a bacterial cell wall extract as a nonspecific immunostimulant to treat topical disorders and to enhance wound healing.

BACKGROUND OF THE INVENTION

2 5 Topical disorders in an animal or human are difficult to treat. Various medicaments have been used to treat various topical disorders with limited success. The safety and effectiveness of these medicaments are variable and depend, in large part, on the route of administration and on the type and severity of the topical disorder. Therefore, what is needed is a therapeutic agent that is safe and effective for treating a variety of topical disorders and that can be administered by a variety of routes.

3 0 Wound healing in an animal or human also is difficult to treat and various medicaments have been used both for the

3 5

5 same and for different types of wounds. The safety and effectiveness of these medicaments are variable and depend, in large part, on the route of administration and on the type and severity of the wound. In horses, healing of wounds on the
10 distal portion of the limbs is associated with difficulties in wound expansion, wound contraction, excessive granulation and prolonged healing time. Therefore, what is needed is a therapeutic agent that is safe and effective for treating a variety of wounds, that reduces wound expansion, enhances wound contraction, prevents excessive granulation, accelerates healing time and that can be administered by a variety of routes.

SUMMARY OF THE INVENTION

15 As used herein, the term "topical" includes the skin, mucous membranes and epithelial cell layers of an animal or human. As used herein, the term "disorder" means a disturbance or abnormality in regular or normal structure or function.

20 A method for preventing, treating and eliminating a topical disorder and for treating a wound in an animal is provided. In accordance with the method, a bacterial cell wall extract is administered to the animal in a sufficient amount to treat the topical disorder or symptoms thereof or to enhance
25 wound healing. The bacterial cell wall extract is safe, has minimal or no adverse side effects, and can be administered to animals such as, but not limited to, mammals, including humans; birds; fish; amphibians; and crustaceans that are suffering from a topical disorder or wound.

30 As the immune response is related to the whole body and is modulated and affected by many complex interactions, nonspecific immune stimulation is capable of accelerating and amplifying many immune responses. Preparations of, but not limited to, yeast, bacterial, viral, plant, biotechnological and
35 chemical origin are capable of non-specifically stimulating the

immune system. Any bacterial species can be used to prepare the bacterial cell wall extract. Extracts from, but not limited to, *Mycobacterium*, *Corynebacterium*, *Proprionebacterium*, *Nocardia*, *Rhodococcus*, *Bordetella*, *Listeria*, and *bacille Calmette-Guerin* (BCG) are preferred. Extracts from *Mycobacteria* and *Corynebacteria* are more preferred. Extracts from *Mycobacterium phlei* are most preferred.

The bacterial cell wall extract can be administered by routes known to those skilled in the art including, but not limited to, topical, oral, nasal, rectal, urethral, intravaginal, intrauterine, intravenous, subcutaneous and intramuscular administration.

When administered to an animal, the bacterial cell wall extract is effective as a therapeutic agent in treating topical disorders such as skin mucous membrane and epithelial diseases including, but not limited to, conditions of the epidermis, dermis, eye, nasopharynx, gastrointestinal tract and urogenital tract. Such diseases include, but are not limited to, gingivitis, psoriasis, decubitus ulcer, dermatitis, allergic dermatitis, actinic keratosis, acne, sinusitis, tonsillitis, chronic buccal ulceration, gastric ulceration, inflammatory bowel disease, chronic gastrointestinal diseases, chronic urogenital diseases, and fungal, yeast, staphylococcal and other bacterial, viral and fungal dermatitides.

Administration of the bacterial cell wall extract is also effective in treating wounds such as, but not limited to, burns, bruises, scrapes, cuts, lacerations and excisional and full-thickness wounds. The bacterial cell wall extract accelerates fibrinization, granulation, contraction and epithelialization of the wound.

Briefly, the bacterial cell wall extract is prepared as follows. Bacteria are grown in liquid medium and harvested. The cell walls are prepared by disrupting the bacteria and then harvesting the disrupted bacteria by centrifugal sedimentation. The cell wall fraction (pellet from the centrifugation step) is

deproteinized by digestion with proteolytic enzymes, treated with detergents, washed, and lyophilized. This fraction can be adsorbed to lipid droplets suspended in an appropriate adjuvant/stabilizer for administration by a variety of routes to an animal suffering from a topical disease or a wound. Preferably the bacterial cell wall extract is formulated in an oil in water emulsion, a water in oil emulsion, a physiologically acceptable buffer, a lotion, and a cream.

The administration of the bacterial cell wall extract described herein differs from conventional therapy in that it nonspecifically causes the immune system to be activated. This enhances the defense capabilities of the immune system, which then acts to prevent, treat and eliminate a variety of topical disorders and accelerates wound healing. Thus, the bacterial cell wall extract is effective in treating topical diseases in an animal that does not have antibodies to the infecting organism. The bacterial cell wall extract also is effective in treating wounds to promote healing regardless of the cause of the wound.

Accordingly, it is an object of the present invention to provide a method effective for preventing a topical disorder.

Another object of the present invention is to provide a method that is effective for treating an ongoing topical disorder.

Another object of the present invention is to provide a method that is effective for preventing reoccurrence of a topical disorder.

Another object of the present invention is to provide a method that is effective in eliminating a topical disorder.

Another object of the present invention is to provide a method that is effective for promoting wound healing.

Another object of the present invention is to provide a method that is effective for preventing infection within a wound.

Another object of the present invention is to provide a method that causes minimal or no adverse side-effects in the recipient.

5 Another object of the present invention is to provide a method that is non-toxic to the recipient.

Another object of the present invention is to provide a method that does not sensitize the recipient to tuberculin skin tests.

10 Another object of the present invention is to provide a method agent that will not cause anaphylaxis in the recipient.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

15

DETAILED DESCRIPTION OF THE INVENTION

A method for preventing, treating or eliminating a topical disorder and for promoting wound healing in an animal is described herein. In accordance with the method of the present invention, a bacterial cell wall extract is administered to an animal exhibiting a topical disorder or having a wound in an amount sufficient to prevent, reduce or eliminate the topical disorder or to promote the healing of the wound. The bacterial cell wall extract stimulates the immune system of an animal in such a way as to cause the body to neutralize or abort the topical disorder and to accelerate wound healing. The bacterial cell wall extract can be administered to animals such as, but not limited to, humans and other mammals, birds, fish, amphibians, and crustaceans to treat or prevent topical disorders and to enhance wound healing. The bacterial cell wall extract can be administered by routes known to those skilled in the art including, but not limited to, topical, oral, nasal, rectal, urethral, intravaginal, intrauterine, subcutaneous, intravenous and intramuscular administration.

20

25

30

When administered to an animal, the bacterial cell wall extract is effective as a therapeutic agent in treating topical disorders such as skin and mucous membrane diseases including, but not limited to, conditions of the epidermis, dermis, eye, nasopharynx, gastrointestinal tract and urogenital tract. Such diseases or disorders include, but are not limited to, gingivitis, psoriasis, decubitus ulcer, dermatitis, allergic contact dermatitis, actinic keratosis, acne, sinusitis, tonsillitis, chronic buccal ulceration, gastric ulceration, inflammatory bowel disease, chronic gastrointestinal diseases, chronic urogenital diseases, burns and fungal, yeast, staphylococcal and other bacterial (viral and fungal) dermatitides. The bacterial cell wall extract can reduce the severity of the adverse symptoms of the topical disorder and, also, can reduce their reoccurrence.

Administration of the bacterial cell wall extract is also effective in treating wounds such as, but not limited to, burns, bruises, scrapes, cuts, excisional and full-thickness wounds. The bacterial cell wall extract accelerates fibrinization, granulation, contraction and epithelialization of the wound. Treatment of the wound in accordance with the method described herein promotes rapid healing and minimizes or eliminates adverse complications including, but not limited to, infection and scarring.

The method described herein differs from conventional therapy in that it nonspecifically stimulates or causes activation of the immune system of the animal having the topical disorder or wound. This immune system activation enhances the defense capabilities of the immune system, thereby ameliorating the topical disorder and accelerating wound healing. Thus, the method is effective in treating topical diseases in an animal that was not previously exposed to and does not already possess antibodies to the disease.

The method of the present invention does not cause a positive tuberculin reaction in the recipient, rarely causes an

anaphylactic response even upon repeated administration of the bacterial cell wall extract, and has minimal or no adverse side-effects. It is to be understood that administration of the bacterial cell wall extract is not an immunization process, but is a process for generally stimulating the immune system so the recipient's own immune system can eliminate the disease or heal the wound. Thus, the treatment method is ideally suited for treatment of ongoing topical disorders and for promoting wound healing and provides a novel method in which conventional medicaments are not utilized.

Bacterial Cell Wall Extract Preparation

Any bacterial species can be used to prepare the bacterial cell wall extract including, but not limited to, *Mycobacterium*, *Corynebacterium*, *Proprione-bacterium*, *Nocardia*, *Rhodococcus*, *Bordetella*, *Listeria*, and *bacille Calmette-Guerin* (BCG). Cell wall extract from mycobacteria and rhodococci are preferred. Cell wall extract from *Mycobacterium phlei* is most preferred. The preferred method for producing the bacterial cell wall extract is described in U.S. Patent No. 4,744,984, which is incorporated herein by reference. Mycobacterial cell wall extract may be commercially obtained from biological supply companies such as Bioniche, Inc. (London, Ontario).

Briefly, the bacterial cell wall extract is prepared as follows. Bacteria are grown in liquid medium and harvested. The cell walls are prepared by disrupting the bacteria and then harvesting the disrupted bacteria by centrifugal sedimentation. The cell wall fraction, which is the pellet from the centrifugation step, is deproteinized by digestion with proteolytic enzymes, treated with detergents, washed, and lyophilized. This fraction can be adsorbed to lipid droplets suspended in an appropriate adjuvant or stabilizer prior to administration to an animal suffering from a topical disorder or wound.

5 The bacterial cell wall extract is preferably emulsified in an adjuvant prior to use. The adjuvant can be any one of many adjuvants known to those skilled in the art. The preferred adjuvant is an oil and water emulsion, which can be prepared by mixing the bacterial cell wall extract with oil, adding an aqueous buffer with detergent, and emulsifying the mixture by any one of several methods known to those skilled in the art. These methods include, but are not limited to, homogenization in a high-speed blender or Potter-Elvehjem homogenizer, 10 sonication and microfluidization. In addition, the bacterial cell wall extract can be emulsified in a number of oils including, but not limited to, mineral oil (Drakeol 6-VR, Penreco, Butler, PA), squalane, squalene and the synthetic mineral oil n-hexadecane. It will be understood by those skilled in the art 15 that the method of preparing the emulsion is not critical. Numerous variations of the composition of the oil and aqueous phases, their proportions and means of emulsification will be apparent to those skilled in the art and can be used with the bacterial cell wall extract in practicing the present method.

20 The preferred emulsions of bacterial cell wall extract are prepared by addition of between approximately 5 g and 15 g of dry, deproteinized bacterial cell wall to a dry, one-liter beaker. Mineral oil, squalene, squalane, or n-hexadecane is added at between approximately 10 ml and 50 ml per gram of 25 cell walls. The suspension is covered and mixed for approximately 30 minutes to overnight. Approximately 10 ml aliquots of the cell wall/oil mixture are transferred to one liter beakers. Five hundred ml of sterile phosphate buffered saline (PBS) is added to each aliquot. Aliquots of approximately 6 30 ml to 7 ml of the mixture are homogenized by microfluidization using a Microfluidics Tabletop Microfluidizer™ model M-110Y at approximately 20,000 psi to 30,000 psi for one flow-through, transferred to sterile bottles, and stored at 4° C.

Optionally, aluminum hydroxide stabilizer may be added to the bacterial cell wall extract emulsion. Aluminum hydroxide is obtained as a 9.4% compressed gel from the Reheis Chemical Co. (Berkeley Heights, NJ) and is hydrated to 1.3% aluminum oxide by the addition of deionized water. The gel is sterilized in an autoclave at 120°C. for 20 minutes before it is added to the bacterial cell wall extract emulsion. One liter of the final emulsion contains about 900 ml of emulsified bacterial cell wall extract, 50 ml of 1.3% aluminum oxide and 40 ml of added PBS. Thimerosal™ (ethylmercurithio-salicylate, Sigma Chemical Co., St. Louis, MO) and antibiotics including, but not limited to, gentamycin and amphotericin B can be added as a preservative to the bacterial cell wall extract emulsion. The preferred concentration of Thimerosal™ is about 0.1 g per liter, of gentamycin about 30 µg/ml and of amphotericin B about 2.5 µg/ml.

Known active ingredients of the bacterial cell wall extract to be administered in the present method include the family of muramyl dipeptides and trehalose dimycolate, as well as any unknown active components which may be present in the deproteinized cell wall skeletons of bacteria. The present invention is effective in treating any topical disorder and in promoting the healing of any wound in which the immune components of the body are present including, but not limited to, neutrophils, lymphocytes and macrophages. Further, it is thought that the bacterial cell wall extract acts on the cells of the immune system to stimulate increased production of cytokines.

Bacterial Cell Wall Extract Formulation and Administration

The bacterial cell wall extract can be provided as a pharmaceutically acceptable composition using formulation methods known to those skilled in the art. Examples of formulation methods may be found in, for example, H.C. Ansel, *et al.*, PHARMACEUTICAL DOSAGE FORMS AND DRUG

DELIVERY SYSTEMS, 6th edition (Williams & Wilkins, Philadelphia 1995), incorporated herein by reference. Other formulations known to those skilled in the art also can be used. The formulations include, but are not limited to, those suitable for oral, ophthalmic, (including intravitreal or intracameral) nasal, topical (including buccal and sublingual), vaginal, rectal, urethral, or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intratracheal, and epidural) administration.

The formulations may be conveniently presented in unit dosage forms prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the pharmaceutical carriers or excipients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The method can be used with any one, all, or any combination of ingredients regardless of the carrier/vehicle used to present them to the responsive immune cells including, but not limited to, carriers such as liposomes, various non-degradable polymers and osmotic minipumps. In addition, the combinations may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired. The biodegradable polymers and their use are described, for example, in Brem, *et al.* 1991. *Journal of Neurosurgery*, 74:441-446, which is incorporated by reference herein.

Formulations of the present method suitable for oral administration may be presented as discrete units including, but not limited to, capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; powders or granules; solutions or suspensions in an aqueous liquid or a

non-aqueous liquid; oil-in-water liquid emulsions or water-in-oil emulsions and as a bolus.

5 A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine. The active ingredient, in a free-flowing form such as a powder or granule, optionally may be mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by
10 molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

15 Formulations of the present method suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin, or sucrose and acacia;
20 and mouthwashes comprising the active ingredient to be administered in a suitable liquid carrier.

Formulations of the present method suitable for topical administration to the skin may be presented as emulsions, ointments, creams, gels, lotions and pastes comprising the
25 active ingredient to be administered in a pharmaceutically acceptable carrier.

Formulations of the present method for rectal administration may be presented as a suppository comprising the active ingredient with a suitable base, for example, cocoa
30 butter or a salicylate.

Formulations of the present method suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns, which is administered in the manner in which
35 snuff is administered, *i.e.*, by rapid inhalation through the

nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations of the present method suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions that may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, in sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) conditions requiring only the addition of a sterile liquid carrier, for example, water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

The optimal dose of the bacterial cell wall extract to be administered varies with the size of the animal that is being treated and with the method of administration. Only an amount sufficient to stimulate the immune system is required. A single dose is from about 0.01 to 10 mg bacterial cell wall extract/ml, more preferably from about 0.05 to 6 mg bacterial cell wall extract/ml and most preferably from about 0.1 to 4.0 mg bacterial cell wall extract/ml. The bacterial cell wall extract is administered in a total volume of from about 0.01 to 10 ml, more preferably from about 0.05 to 7.5 ml and most preferably from about 0.1 to 5.0 ml. For topical use, the

bacterial cell wall extract is administered as an emulsion or is suspended in a lotion, cream, gel, or other carrier known to one skilled in the art.

5 The bacterial cell wall extract of the present invention can be administered one time or several times to the same recipient. The dosage amount and the dosage schedule can be determined readily by those skilled in the art. Preferred dosage formulations are those containing a dose or unit, subdose or subunit, or fraction thereof of the bacterial cell
10 wall extract in, but not limited to, the formulations described herein. Further, it should be understood that in addition to the ingredients particularly mentioned herein, the present method may include other agents conventional in the art having regard to the type of formulation be used.

15 The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof.

EXAMPLE 1

20 PREPARATION OF BACTERIAL CELL WALL EXTRACT

The preparation of *M. phlei* cell wall extract, as outlined in Example 1, is representative of the preparation of cell wall extract from other bacterial species.

25 *Mycobacterium phlei* was obtained from the Institut fur Experimental Biologie and Medizin, Borstel, West Germany, and was stored as a suspension in sterile milk at -60°C. Approximately eleven transfers of the isolate were made between 1976 and 1985 without any diminution of biological activity of the modified cell walls. The *M. phlei* was cultured
30 on Petragrani medium (Difco Labs, Detroit, MI).

Mycobacterium phlei cell walls were prepared with a Heat System sonicator (previously called a Branson sonicator) Model XL2015. Approximately 400 grams of moist cell mass was placed into a clean blender with a capacity of
35 approximately 1200 ml. The cell mass was mixed at high speed

for between 30 to 60 seconds. After mixing, 6 ml of Tween 80 and between 200 and 400 ml of sterile water were added to the cell mixture. The entire cell suspension was then mixed in the blender at low speed for about ten seconds.

5 Cell disruption was accomplished by ultrasonic cell disruption using a Heat System sonicator and a 3/4 tapped horn. Five hundred milliliters of a cell suspension, wherein the cells comprise about 50% to 70% of the volume, were placed in a one liter beaker and sonicated at a setting of eight
10 for about five minutes. The sonicate was stored in a sterile flask on ice during the fractionation process.

The sonicate was transferred to 250 ml centrifuge bottles and centrifuged for one hour at 27,500 x g at 15°C. in an intermediate speed centrifuge with a GSA rotor. The
15 supernatant fluid from the centrifugation was decanted and discarded. The undermost, white pellet of unbroken cells was discarded. The sedimented crude cell wall fraction was transferred to a blender and suspended in sterile, deionized water by mixing at low speed. The crude cell wall fraction
20 was washed by resuspension and centrifugation at 27,500 x g at 15°C. for one hour). Again, the undermost, white pellet of unbroken cells was discarded.

After washing the crude cell wall fraction, the pellet was resuspended in sterile, deionized water and spun for five
25 minutes at 350 x g to sediment unbroken cells while retaining the cell walls in the supernatant fluid. The supernatant fluid was decanted and centrifuged at 27,500 x g for 1 hour at 15°C. to sediment the crude cell wall fraction.

The crude cell wall fraction was deproteinized by
30 digestion with proteolytic enzymes. The crude cell wall fraction, derived from about 400 g of whole cells, was resuspended in 1 liter of 0.05 M Tris-HCl, pH 7.5, by mixing at low speed. After the crude cell wall fraction was thoroughly resuspended in the Tris buffer, 50 mg of trypsin
35 (pancreatic trypsin, Sigma Chemical Co., St. Louis, MO) were

added and the suspension was stirred using a magnetic stirring bar at 35°C for six hours. Following trypsin treatment, 50 mg of pronase (*Streptomyces griseus* protease, Sigma Chemical Co., St. Louis, MO) were added to each liter of trypsin treated cell wall suspension. The suspension was stirred using a magnetic stirring bar for 12 to 18 hours at 35°C.

The protease digested cell wall fraction was treated with detergent and phenol. To each liter of cell wall suspension, 60 g of urea (J. T. Baker Chemical Co., Phillipsburg, NJ), 2.0 ml of 100% Triton X-100 (polyoxyethylene ethers, Sigma Chemical Co., St. Louis, MO), and 100 g of phenol crystals (Fisher Scientific, Fair Lawn, NJ) were added. The flask containing the suspension was covered loosely with aluminum foil, warmed to 60°-80°C. and stirred for one hour. The deproteinized cell wall fraction was spun for 10 minutes at 16,000 x g in a GSA rotor. The supernatant fraction was decanted and discarded and the dark fluid beneath the pellet was removed using a disposable pipette. The cell wall pellet was washed 3 times by resuspension in about one liter of sterile water and centrifuged at 16,000 x g for 10 minutes in a GSA rotor.

The washed, modified mycobacterial cell wall extract (MCWE) or cell wall pellet was lyophilized by transferring the suspension to a lyophilizing flask with a small amount of deionized sterile water. One 300 ml lyophilizing flask was used for each 30 grams of wet cell wall starting material. The cell wall suspension was shell frozen by rotating the flask in ethanol that had been cooled with solid carbon dioxide. After the content of the flask was frozen, the flask was attached to a lyophilization apparatus (Virtis Co., Inc., Gardiner, NY) and lyophilized. After lyophilization, the sample was transferred to a sterile, screw-cap container and stored at -20°C in a desiccator jar containing anhydrous calcium sulfate.

EXAMPLE 2
EMULSIFICATION OF BACTERIAL CELL WALL
EXTRACT

5 Emulsions of a mycobacterial cell wall extract (MCWE)
were prepared in four steps: (1) addition of dry,
deproteinized, mycobacterial cell wall extract and squalane to
an emulsification vessel, (2) suspension of the cell wall extract
in the oil, (3) addition of buffered saline solution containing a
10 detergent to the mixture of cell wall extract and oil, and (4)
emulsification of the oil-cell wall extract complex into the
aqueous detergent saline solution.

Emulsification was accomplished by microfluidization
using a Microfluidics tabletop Microfluidizer™ Model M-
110Y at 10,700-23,000 psi for one flow-through. The typical
15 volume run was six to seven liters per run. Several grams of
lyophilized MCWE (prepared as described in Example 1,
above) were added to a dry, sterile, one liter beaker. Squalane
was added at a concentration of 20 ml per gram of MCWE and
the mixture was covered and allowed to sit overnight. The
20 optimum concentration of oil in the oil and water suspension is
between approximately 1% and 7%. Ten ml aliquots of the
oil-MCWE mixture were transferred to sterile one liter
beakers. Five hundred milliliters of sterile, phosphate
buffered saline (0.05M sodium phosphate, pH 7.2, 9 g NaCl
25 and 2 ml Tween-80 per liter of deionized water) were added to
each 10 ml aliquot of oil-MCWE. The mixture was
homogenized by microfluidization and transferred to sterile,
capped bottles for storage at 4°C. Samples of the emulsion
were examined under a cover-slip with a light microscope to
30 confirm that the oil droplets were small and granular in
appearance rather than clear with dark borders. Granular
appearing droplets indicate proper adsorption of the cell wall
to the oil carrier.

Bottles of the cell wall preparation in oil-in-water
35 emulsion were pooled in a sterile mixing vessel, and 30 µg/ml

gentamycin and 2.5 µg/ml amphotericin B were added to each liter of cell wall emulsion as preservatives. Sterile Type I glass vials and plastic syringes were filled with from 1.5 ml to 10 ml of the stabilized emulsion under sterile laminar air flow using a Filamatic Vial Filler (National Instrument Co., Baltimore, MD). The vials and syringes were capped, sealed and stored at 4°C.

EXAMPLE 3

1 0 TREATMENT OF HERPES SIMPLEX WITH TOPICAL MCWE

In this study, four patients with herpes simplex (cold sores) were treated with the MCWE emulsion (described above in Example 3) containing 1 mg MCWE/ml. Outcome criteria for this study relate to resolution of symptoms and reoccurrence of the cold sores.

SUBJECT 1-PATIENT GM

GM, a male, has a history of reoccurring cold sores. Non-prescription medications provided minimal resolution of his symptoms and no decrease in reoccurrence of the cold sores. A thin film of MCWE emulsion was applied directly to the cold sores two to three times daily. GM reported rapid resolution of the cold sores and a decrease in their reoccurrence to once a year or less. He also reported that the application of topical MCWE to the cold sore area, whenever he felt a cold sore starting, resulted in rapid resolution of the cold sore. No adverse side effects of topical MCWE were reported.

SUBJECT 2-PATIENT GO

3 0 Patient GO, a female, has a history of reoccurring cold sores which are stress related. Non-prescription medications had no affect on her cold sores or on their reoccurrence. A thin film of MCWE emulsion was applied directly to her cold sores two to three times daily. GO reported resolution of the cold sores within three days and a decrease in their

reoccurrence. No adverse side effects of topical MCWE were reported.

SUBJECT 3-PATIENT SM

5 Patient SM, a female, has a history of reoccurring cold sores. Treatments, including 5% acyclovir ointment, had a minimal effect on her cold sores and no effect on their reoccurrence. A thin film of MCWE emulsion was applied directly to her cold sores two to three times a day. SM reported resolution of the cold sores and a decrease in their reoccurrence. She also reported that using MCWE emulsion topically when cold sores first started prevented their emergence. No adverse side effects of topical MCWE were reported.

SUBJECT 4-PATIENT RB

1 5 Patient RB, a male, has a history of cold sores, fever blisters and lesions on and around his lips. MCWE emulsion was applied directly to the affected area for three days. RB reported drying and regression of the lesions within 24 hours and complete resolution of the lesions after 72 hours. No adverse side effects of topical MCWE were reported.

SUMMARY

2 5 The data presented above show that treatment of herpes simplex (cold sores) with topical MCWE results in a resolution of the symptoms of the cold sores and a decrease in the reoccurrence of the cold sores. Further, these data show that application of topical MCWE causes no or minimal side effects.

3 0

EXAMPLE 4

TREATMENT OF DERMATITIS WITH TOPICAL MCWE

3 5 In this study, ten patients with dermatitis were treated with a topical MCWE emulsion containing 1 mg MCWE/ml or with MCWE microemulsion containing 100 mg MCWE in 1

5 ml of Beltapharm cream containing 18 g tefose 63, 8 g isostearyl isostearate, 6 g labrafil M 1944, 0.05 g viox, 0.10 g nipagin, 0.02 g nipasol, 20 g glycerol and purified water to 100 grams (Belapharm, Milan, Italy). Outcome criteria for this study relate to resolution of symptoms and reoccurrence of the dermatitis.

Patient 1-JM

10 JM, a 25 year old male, reported contact dermatitis (a rash) on his right arm caused by chemical exposure. A thin layer of the MCWE emulsion was applied directly to the rash three times in one day. JM reported resolution of the rash within 24 hours and no reoccurrence of the dermatitis. No adverse side effects of topical MCWE were reported.

Patient 2-MH

15 MH, an 18 month old male, had dermatitis (a rash) due to allergic reaction to detergent. The use of topical cortisone cream in combination with liquid antihistamine over a two week period had no effect. A thin layer of MCWE emulsion was applied to the rash two times a day for seven days. The redness and inflammation disappeared within 48 hours. However, the "itchiness" did not resolve until three days after completion of the treatment. There was no recurrence of the dermatitis. No adverse side effects of topical MCWE were reported.

25

Patient 3-MF

30 Patient MF, an 18 year old female, has a history of severe acne precipitated by stress. The use of Vitamin A and antibiotics were ineffective. MCWE emulsion was applied directly to the acne two times per day. MF reported that the lesions dried up and flaked off within 24 hours. No adverse side effects of topical MCWE were reported.

Patient 4-JK

35 Patient JK, a female, had a three year history of dermatitis (a rash) on her hands. Treatment with topical

administration of a steroid cream provided no relief. A thin layer of the MCWE emulsion was applied directly to her hands one time a day for seven days. Her condition cleared within the seven days. JK reported that she has treated several recurrences of the rash with topical MCWE with subsequent resolution of the symptoms. No adverse side effects of topical MCWE were reported.

Patient 5-JT

Patient JT, a 30 year old female, was diagnosed with a dermatitis (a rash) caused by contact with detergents and chemicals which caused cracking and bleeding of the skin on her hands. Treatment with hydrocortisone cream three times daily for two weeks provided minimal relief. MCWE cream was applied directly to her hands two times a day for seven days. Initially improvement was minimal. After discontinuation of the topical MCWE, the rash began to resolve and did not reoccur for six weeks. JT reported that when the rash did reoccur, application of topical MCWE alleviated the condition for at least another six weeks. JT reported having used topical MCWE on at least eight occasions. The only adverse side effect was a burning sensation where MCWE cream was applied to the rash.

Patient 6-HB

Patient HB, a female, has eczema which occurs during periods of high stress and after ingestion of certain foods and/or contact with detergents. The use of cortisone based creams and other cortisone treatments provided minimal relief. A thin layer of MCWE cream was applied to the eczema one time a day for seven days. The topical MCWE arrested the progression of the eczema and prompted rapid healing of the eczema. HB reported use of topical MCWE on at least twelve subsequent occasions with no adverse side effects.

Patient 7-RW

5 Patient RW, a male, had a severe dermatitis that presented as puritic, erythematous, scaly lesions over his entire body. He spent one month at the Mayo Clinic in Jacksonville, FL with no satisfactory result. He was diagnosed as having a contact dermatitis resulting from an allergic reaction to preservatives. Treatment with topical application of a locally prepared pharmaceutical cream provided minimal resolution of his dermatitis. MCWE emulsion mixed into the locally prepared pharmaceutical cream (100g MCWE/ml cream) was applied directly to the dermatitis and his condition improved within hours. RW reported unchanged frequency in the recurrence of his dermatitis. However, use of topical MCWE cream continued to alleviate his symptoms within hours. No adverse side effects of topical MCWE cream were reported.

Patient 8-DT

20 Patient DT, a three year old male, had a dermatitis-like rash on his cheeks during cool, dry weather. The affected areas were treated with a thin layer of MCWE cream one time a day for two days. The topical MCWE provided immediate relief and the rash disappeared within 24 hours. No adverse side effects of topical MCWE were reported.

Patient 9-BT

25 Patient BT, an 18 month old male, had an extensive raised red rash on his buttocks. The affected area was treated with a thin layer of MCWE cream one time a day for three days. The treated area became inflamed and treatment was discontinued and the inflammation subsided. No subsequent effects of the treatment were reported. There is explanation available for this adverse reaction to topical MCWE treatment.

Patient 10-SA

35 Patient SA, a 50 year old male, had a 1 cm diameter actinic keratosis lesion on the left upper cheek at the hair line. The affected area was treated with 1 mg MCWE in 1 ml of Beltapharm cream two times a day for fourteen days. SA

reported that the lesion began to dry and had completely flaked-off by 25 days. The lesion healed completely with no resultant scarring. The lesion has not recurred during the last three years. No adverse side effects of MCWE were reported.

5

SUMMARY

These data show that treatment of dermatitis with topical MCWE results in a resolution of the symptoms of the dermatitis and a decrease in the reoccurrence of the dermatitis in 9 out of the 10 patients treated. Further, these data show that application of topical MCWE cause no or minimal side effects in 9 of the 10 patients treated.

10

EXAMPLE 4-

TREATMENT OF WOUNDS WITH TOPICAL MCWE

15

In this study, five patients with five different types of wounds were treated with an MCWE emulsion containing 1 mg MCWE/ml or with MCWE microemulsion containing 100g or 500g MCWE in 1 ml of cream as described in Example 3. Outcome criteria for this study are related to the time necessary for healing of the wound, to the occurrence of infection within the wound and to amount of scar tissue left after the wound healed.

20

Patient 1-GH

Patient GH, an elderly male, had very large bed sores. Use of antibiotic cream and oral antibiotics were ineffective. A layer of MCWE cream (100g/ml) was applied directly to the bed sores two times a day. GH reported that a marked improvement in healing of the bed sores occurred within four days. No adverse side effects of topical MCWE were reported.

30

Patient 2-KP

Patient KP, a male, sustained a large gash on one leg in a bicycle accident. MCWE cream (100g/ml) was applied directly to the gash two times a day for seven days. KP reported complete healing of the wound with no infection and

35

with no scarring. No adverse side effects of topical MCWE were reported

Patient 3-MF

5 Patient MF, a male, sustained a burned lower lip in a soldering accident. MCWE cream (100g/ml) was applied directly to the burned lip three times a day for fourteen days. MF reported complete healing of the lip with no infection and with no scarring. No adverse side effects of topical MCWE were reported.

1 0

Patient 4-IR

Patient IR, a female, was diagnosed with shingles on her forehead and scalp. Treatment with 500 mg oral cephalex four times per day for five days had no effect. On day 6 her eyes and cheeks began to swell and her lesions became sensitive to touch. On day 13, IR took one oral dose of MCWE and began applying MCWE cream, (500 g/ml) to her shingles two times a day. On day 15, the lesions broke open and drained and their sensitivity to touch decreased. On day 18, IR developed a headache and flu-like symptoms. On day 19, the headache and flu-like symptoms disappeared. On day 20, IR stopped topical MCWE. On day 25 the swelling in her eyelids and cheeks were gone and most of the lesions had resolved.

1 5

2 0

Patient 5-VO

2 5 Patient VO, a male, had a progressive ulcerative lesion covering his entire lower lip which was biopsied and diagnosed as a bacterial infection. VO's lesion did not respond to six weeks of treatment with topical antiviral and antibacterial drugs, either individually or in combination. Topical treatment with MCWE emulsion (1 mg/ml) was begun approximately six weeks after the lesion first appeared. The lesion began to recede at its margins and to decrease in size. After two weeks of topical MCWE, the lesion was one-third its original length and width. VO has myasthenia gravis, which was not aggravated by topical MCWE.

3 0

3 5

SUMMARY

These data show that treatment of wounds with topical MCWE results in healing of the wounds. Further, these data show that application of topical MCWE causes no or minimal side effects.

EXAMPLE 5

TREATMENT OF WOUNDS WITH SYSTEMIC MCWE

In this study, eight healthy adult horses (ages 3-12) were anesthetized and a 2.5 cm² full-thickness wounds were created surgically on the dorsolateral aspect of the metacarpus proximal to the fetlock joint of the fore limb and from the contralateral metatarsal region of the rear limb of each animal (N=16). The wounds were bandaged and the horses were allowed to recover from the anesthesia. Six hours post-surgery, 4 of the 8 horses were injected intravenously with 1.5 mg MCWE emulsion (experimental), prepared as described in Example 2. The other 4 horses received an equal volume of saline (control). The bandages were removed one day post-surgery, cleansed with sterile saline and rebandaged. Subsequently the bandages were changes every two days until the wounds healed.

Total wound area, epithelialization + nonepithelialized area and nonepithelialized area were measured using planar morphometry of digitized photographs. Outcome criteria for this study were total wound area, healing due to contraction and healing due to epithelialization of the 6.25 cm² wound. Complete healing was defined as complete epithelialization. Although horses do show individual variation in wound healing, previous studies have shown that complete healing requires approximately 60 days

Control Wounds

At day 5 post-surgery, the 8 control wounds showed a nice even fibrin layer. At day 15 post-surgery, the control

wounds continue to heal nicely. At day 27 post-surgery, the control wounds continued to heal nicely. At day 18 post-surgery, the control wounds had not contracted. By day 25 post surgery, the control wounds had contracted by 30%. At day 41 post-surgery, the control wounds continued to heal nicely, but none had healed completely. At day 56 post-surgery, 5 of the 8 control wounds in the 4 untreated horses had not healed completely. In other experiments with untreated controls, although there was individual variation, complete healing required approximately 60 days.

Treated Horses

At day 5 post-surgery, the 8 MCWE treated wounds showed a nice even fibrin layer. At 15 days post-surgery, the wounds continued to heal nicely. At day 18 post surgery, the MCWE treated wounds had contracted by an average of 30%. At day 27 post-surgery, the MCWE treated wounds continued to heal nicely and the wounds on two of the horses had almost completely healed. At day 38, post surgery, the wounds on 1 horse had completely healed and the wounds on the other 3 MCWE treated horses were almost completely healed. At day 41 post-surgery, 2 of the MCWE treated horses were completely healed and the other 2 were almost completely healed. At day 56 post-surgery, 8 of the 8 wounds in the 4 MCWE treated horses were completely healed.

Summary

Variability of wound healing were not affected by age or gender. Within in the initial 72 hors post-surgery, experimental group horses had reduced wound expansion (6.63 cm²) as compared to control group horses (7.31 cm²). Further, experimental group horses showed increased wound healing by wound contraction (P=0.066) and by a more rapid decrease in nonepithelialized surface area (P=0.058). At day 36 post-wound, experimental horses had a mean total wound

area of 2.28 cm², whereas control horses had a mean total wound area of 2.84 cm² and a nonepithelialized mean area of 0.78 cm² and 1.43 cm².

5 These data show that treatment of wounds with systemic MCWE results in decreased wound expansion, increased wound contraction and to decreased healing time. Further, these data show that systemic delivery of MCWE results in no adverse side effects.

EXAMPLE 6
PREPARATION OF *RHODOCOCCUS EQUI* CELL WALL
EXTRACT (RCWE)

15 *R. equi*, a coryneform organism previously designated as *Corynebacterium equi*, cell wall extract is prepared as in Example 1.

EXAMPLE 7

IMMUNOSTIMULATORY PROPERTIES OF RCWE

Thirty-two CD1 outbred mice were injected intraperitoneally with 200 µg of RCWE in 0.5 ml of 2% oil in normal saline emulsion (RCWE mice). Sixteen CD1 outbred mice were injected intraperitoneally with 0.5 ml of normal saline (saline control mice) and 8 CD1 mice were maintained as environmental controls (environmental control mice). After 72 hours, 16 RCWE mice and 8 saline control mice were challenged with 5XLD₅₀ of *Pasteurella multocida* and 16 RCWE mice and 8 saline control mice were challenged with 20XLD₅₀ of encephalomyocarditis (EMC) virus. At 14 days post-challenge, 10/32 RCWE mice, 0/16 saline control mice and 8/8 environmental control mice survived.

30 These data demonstrate that RCWE is a nonspecific immunostimulant.

EXAMPLE 8

STIMULATION OF NITRIC OXIDE PRODUCTION BY RCWE AND BY MCWE

35 Murine macrophage cell line RAW 264.7 cells were
plated in 24 well tissue plates. When the cells formed a

confluent monolayer, media was removed from the wells and replaced with 1 ml of fresh media containing from 5-80 μg of RCWE or 5-80 μg of MCWE. After 24 hours incubation at 37°C in an atmosphere containing 5% CO₂, nitric oxide (NO) production was determined using the colorimetric reaction of Griess (Griess, P. Demerkungen zu der Abhandlung der HH. Wesdlsky und Benedikt, UEBER EINIGE AZOVERDINDUNGEN. Chem. Ber. 12: 426-428, 1987).

1 0

TABLE 1

Mean Nitric Oxide (NO) Production (nmol/10⁶ cells/24h)

SAMPLE	5 μg	10 μg	20 μg	40 μg	80 μg
RCWE ¹	0.0336	0.0986	0.1926	0.2540	0.2773
MCWE ²	0.1650	0.2124	0.2600	0.2910	0.3107

¹ Mean of all samples for RCWE

² Mean of all samples for MCWE

1 5

The data in Table 1 demonstrate that the nonspecific immunostimulant RCWE has nonspecific immunostimulatory properties similar to those of the nonspecific immunostimulant MCWE in promoting the generation of NO by RAW 264.7 cells.

2 0

Bacterial cell preparations of *Propriosebacterium acne* (Eqstim[®], Immunomed, Florida), *Bacillus Calmette-Guerin* (BCG), *Bordetella pertussis* and other *Bortetella spp.* and the rubeolla virus product RVI (Eudaemonic, Omaha, NE) have been used as nonspecific immunostimulants and have nonspecific immunostimulatory properties similar to those of the nonspecific immunostimulants RCWE and MCWE.

2 5

3 0

It should be understood, of course, that the foregoing relates only to a preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the invention as set forth in the appended claims.

We claim:

- 5 1. A method for treating a topical disorder in an animal, comprising administering to the animal suffering from the topical disorder an amount of a nonspecific immunostimulant effective to treat the topical disorder in the animal.
- 10 2. The method of claim 1, wherein the non-specific immunostimulant is a bacterial cell wall extract.
- 15 3. The method of claim 2, wherein the bacterial cell wall extract is selected from the group consisting of mycobacterial cell wall extract and corynebacterial cell wall extract
- 20 4. The method of claim 3, wherein the mycobacterial cell wall extract is a *Mycobacterium phlei* cell wall extract.
- 25 5. The method of claim 3, wherein the corynebacterial cell wall extract is a *Rhodococcus equi* cell wall extract.
- 30 6. The method of claim 4, wherein the cell wall extract is applied in a formulation selected from the group consisting of an emulsion, a cream, a lotion and an ointment.
7. The method of claim 6 wherein the cell wall extract is applied in a formulation comprising a cream.

8. The method of claim 6, wherein the cell wall extract is applied in a formulation comprising an emulsion.
- 5 9. The method of claim 8, wherein the emulsion is an oil and water emulsion.
- 10 10. The method of claim 9, wherein the oil is selected from the group consisting of mineral oil, squalane, squalene and n-hexadecane.
- 15 11. The method of claim 1, wherein the topical disorder is selected from the group consisting of dermatitis, psoriasis, decubitus ulcer, acne, actinic keratosis, buccal ulceration, herpes simplex, and eczema.
- 20 12. A method for preventing the reoccurrence of a topical disorder in an animal having a topical, comprising administering to the animal having the topical disorder an amount of a non-specific immunostimulant effective to prevent the reoccurrence of the topical disorder in the animal.
- 25 13. The method of claim 12, wherein the non-specific immunostimulant is a bacterial cell wall extract.
- 30 14. The method of claim 13, wherein the bacterial cell wall extract is selected from the group consisting of a mycobacterial cell wall extract and a corynebacterial cell wall extract.
- 35 15. The method of claim 14, wherein the mycobacterial cell wall extract is a *Mycobacterium phlei* cell wall extract.

16. The method of claim 14, wherein the corynebacterium cell wall extract is a *Rhodococcus equii* cell wall extract.
- 5 17. The method of claim 15, wherein the cell wall extract is applied in a formulation selected from the group consisting of an emulsion, a cream, a lotion and an ointment.
- 10 18. The method of claim 17, wherein the cell wall extract is applied in a formulation comprising a cream.
- 15 19. The method of claim 17, wherein the mycobacterial cell wall extract is applied in a formulation comprising an emulsion.
- 20 20. The method of claim 19, wherein the emulsion is an oil and water emulsion.
- 25 21. The method of claim 20, wherein the oil is selected from the group consisting of mineral oil, squalane, squalene and n-hexadecane.
- 30 22. The method of claim 12, wherein the topical disorder is selected from the group consisting of dermatitis, psoriasis, decubitus ulcer, acne, actinic keratosis, buccal ulceration, herpes simplex, and eczema.
23. A method for treating a wound in an animal having a topical, comprising administering to the animal having the wound an amount of a non-specific immunostimulant effective to promote the healing of the wound in the animal.

- 24 The method of claim 23, wherein the non-specific immunostimulant is a bacterial cell wall extract.
- 5 25. The method of claim 24, wherein the bacterial cell wall extract is selected from the group consisting of a mycobacterial cell wall extract and a corynebacterial cell wall extract.
- 10 26. The method of claim 25, wherein the mycobacterial cell wall extract is a *Mycobacterium phlei* cell wall extract.
- 15 27. The method of claim 25, wherein the corynebacterium cell wall extract is a *Rhodococcus equi* cell wall extract.
28. The method of claim 23, wherein the cell wall extract is administered systemically.
- 20 29. The method of claim 28 wherein the cell wall extract is an emulsion.
- 25 30. The method of claim 29, wherein the emulsion is an oil and water emulsion.
31. The method of claim 30 wherein the oil is selected from the group consisting of mineral oil, squalane, squalene and n-hexadecane.
- 30 32. The method of claim 30, wherein the oil is squalane.

AMENDED CLAIMS

[received by the International Bureau on 9 August 1998 (09.08.98);
original claims 1-32 replaced by amended claims 1-28 (4 pages)]

1. A method for treating a topical disorder in an animal, comprising administering to the animal in need of treatment for a topical disorder an amount of a bacterial cell wall extract effective to treat the topical disorder in the animal.
- 5 2. The method of claim 1, wherein the bacterial cell wall extract is selected from the group consisting of cell wall extract from *Mycobacterium* species and cell wall extract from *Rhodococcus* species.
3. The method of claim 2, wherein the *Mycobacterium* species is *Mycobacterium phlei*.
- 10 4. The method of claim 2, wherein the *Rhodococcus* species is *Rhodococcus equi*.
5. The method of claim 3, wherein the bacterial cell wall extract is applied in a formulation selected from the group consisting of an emulsion, a cream, a lotion and an ointment.
6. The method of claim 5 wherein the bacterial cell wall extract is applied in a
15 formulation comprising a cream.
7. The method of claim 5, wherein the bacterial cell wall extract is applied in a formulation comprising an emulsion.
8. The method of claim 7, wherein the emulsion is an oil and water emulsion.
9. The method of claim 8, wherein the oil is selected from the group consisting of
20 mineral oil, squalane, squalene and n-hexadecane.

10. The method of claim 1, wherein the topical disorder is selected from the group consisting of dermatitis, psoriasis, decubitus ulcer, acne, actinic keratosis, buccal ulceration, herpes simplex and eczema.

11. A method for preventing the reoccurrence of a topical disorder in an animal, comprising administering to the animal in need of preventing the reoccurrence of the topical disorder an amount of a bacterial cell wall extract effective to prevent the reoccurrence of the topical disorder in the animal.

12. The method of claim 11, wherein the bacterial cell wall extract is selected from the group consisting of cell wall extract from *Mycobacterium* species and cell wall extract from *Rhodococcus* species.

13. The method of claim 12, wherein the *Mycobacterium* species is *Mycobacterium phlei*.

14. The method of claim 12, wherein the *Rhodococcus* species is *Rhodococcus equii*.

15. The method of claim 13, wherein the bacterial cell wall extract is applied in a formulation selected from the group consisting of an emulsion, a cream, a lotion and an ointment.

16. The method of claim 15, wherein the bacterial cell wall extract is applied in a formulation comprising a cream.

17. The method of claim 15, wherein the bacterial cell wall extract is applied in a formulation comprising an emulsion.

18. The method of claim 17, wherein the emulsion is an oil and water emulsion.

19. The method of claim 18, wherein the oil is selected from the group consisting of mineral oil, squalane, squalene and n-hexadecane.

20. The method of claim 11 wherein the topical disorder is selected from the group consisting of dermatitis, psoriasis, decubitis ulcer, acne, actinic keratosis, buccal ulceration, herpes simplex and eczema.

21. A method for promoting the healing of a wound in an animal having a wound, comprising administering to the animal having the wound an amount of a bacterial cell wall extract effective to promote the healing of the wound in the animal.

22. The method of claim 21, wherein the bacterial cell wall extract is selected from the group consisting of cell wall extract from *Mycobacterium* species and cell wall extract from *Rhodococcus* species.

23. The method of claim 22, wherein the *Mycobacterium* species is *Mycobacterium phlei*.

24. The method of claim 22, wherein the *Rhodococcus* species is *Rhodococcus equi*.

25. The method of claim 21, wherein the bacterial cell wall extract is administered systemically.

26. The method of claim 25, wherein the bacterial cell wall extract administered systemically is an emulsion.

27. The method of claim 26, wherein the emulsion is an oil and water emulsion.

28. The method of claim 27, wherein the oil is selected from the group consisting

of mineral oil, squalane, squalene and n-hexadecane.

STATEMENT UNDER ARTICLE 19**Amendments**

More particularly, dependent claims 2, 13, 24 and 32 have been cancelled.

Independent claims 1, 12 and 23 have been amended to include the subject matter of claims 2, 13 and 24 respectively.

Dependent claims 3, 4, 5, 14, 15, 16, 17, 18, 19, 25, 26, 27, 28 and 29 have been amended.

Claims 3 - 12 have been renumbered as new claims 2 - 11.

Claims 14 - 23 have been renumbered as new claims 12 - 21.

Claims 25 - 31 have been renumbered as new claims 22 - 28.

REMARKS

With dependent claims 2, 13, 24 and 32 having been cancelled, the remaining claims have been renumbered consecutively (Administrative Instructions, Section 205(b)).

The above amendments to claims, represented now by claims 1 - 28, more particularly point out the inventive aspect of the method of applicant's invention. The above amendments clarify that applicant's invention is a method for treating a topical disorder in an animal, for preventing the recurrence of a topical disorder in an animal, comprising administering to the animal an amount of a bacterial cell wall extract effective to treat the topical disorder to prevent the reoccurrence of the topical disorder and to treat the wound in the animal.

The amendments to the claims, represented now by claims 1 - 28, are supported throughout the specification and especially at page 2, lines 29 - 34 and at page 5, lines 17 - 26, wherein it is disclosed that administration of bacterial cell wall extract can be administered in an amount sufficient to treat and to prevent the reoccurrence of a topical disorder and to treat a wound in an animal. The amendments to claims 2, 12 and 22 are supported at page 26, lines 13 - 15, wherein it is disclosed that *Rhodococcus equii*, a coryneform organism, was previously designated as *Corynebacterium equii*.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/00297

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K35/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 16727 A (VETREPHARM INC) 4 August 1994 see page 5, line 4-11 see page 5, line 25-33	1-8, 11-19, 22
Y	see page 13, line 30 ---	1-32
X	US 4 744 984 A (RAGLAND WILLIAM L) 17 May 1988 cited in the application	1-4, 11-15, 22
Y	see column 3, line 24; claim 2 -----	1-32



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 June 1998

Date of mailing of the international search report

09/07/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Herrera, S

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 98/00297

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9416727 A	04-08-1994	AU 691797 B	28-05-1998
		AU 5968094 A	15-08-1994
		BR 9406496 A	02-01-1996
		CA 2154689 A	04-08-1994
		CN 1118574 A	13-03-1996
		EP 0681479 A	15-11-1995
		JP 8508976 T	24-09-1996
		US 5759554 A	02-06-1998
US 4744984 A	17-05-1988	AU 605116 B	10-01-1991
		AU 6525886 A	05-05-1987
		CA 1293190 A	17-12-1991
		DE 3684305 A	16-04-1992
		EP 0238657 A	30-09-1987
		WO 8702249 A	23-04-1987